# The Characterization of Salmonella isolated from Pig Meat in Northern Ireland by PFGE and Antibiotic Resistance Profiles

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**Abstract**

The emergence of antibiotic resistance and especially multiple antibiotic resistance *Salmonella* has become a concern for the pig industry throughout the EU. Pig herds and pork are considered as principal reservoirs for the multi-resistant *Salmonella* type Typhimurium DT104, which has acquired resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline. This resistance pattern is also known as ACSSuT. In this study Porcine *Salmonella* strains were isolated between December 2005 and December 2006. The strains originated from an abattoir study sampling the 'oyster' cut. The Antimicrobial Resistance Profiles of all *Salmonella* isolates in this study were determined by disk diffusion tests. Twelve antibiotics were utilized throughout the profiling procedure. Pulsed Field Gel Electrophoresis (PFGE), which is regarded as the "Gold Standard" for the typing and strain identification of *Salmonella* isolates, was used to determine DNA fingerprints of the *Salmonella* isolates using the restriction enzyme *Xba*I (Invitrogen). The fragments were then separated by PFGE in a Chef DR II system (Bio-Rad). This enabled comparison of *Salmonella* isolated in this study.

**Introduction**

There are over 2,300 serotypes of *Salmonella* worldwide (Foley *et al.*, 2001, 2000). In 2005 there were 176 reported cases of human salmonellosis in Northern Ireland. This has been the lowest level reported in the past 12 years, but in 2004 there were three major outbreaks of *Salmonella* in the Northern Ireland involving *S. tyhimurium* DT104, *S. virchow* and *S. newport* (CDSC, 2006). These outbreaks are a reminder of how important it is to monitor and control *Salmonella* in our food animals. Over the past five years *Salmonella typhimurium* has consistently been the second most common cause of human salmonellosis. *S. typhimurium* type DT104 has also been the most commonly isolated *Salmonella* on the island of Ireland and in Great Britain (CDSC, 2006)(Randall *et al.*, 2003) (Foley *et al.*, 2000). There were 33 reported cases of *Salmonella typhimurium* in Northern Ireland alone, of which the most frequently reported phage type was *Salmonella typhimurium* DT104 (CDSC, 2006). It has been established that multi-drug resistant *S. typhimurium* DT104 has acquired antibiotic resistance to ampicillin, chloroamphenicol, streptomycin, sulphonamides and tetracycline's. This resistance profile has been abbreviated to ACSSuT resistance (Alban *et al.*, 2001) (Threlfall, 2000). An abattoir study undertaken in the UK in 2003 to determine the carriage of food borne pathogens in food animals reported the prevalence of *Salmonella* carriage at slaughter was 23.4% for pigs (DEFRA, 2004). Studies in The Netherlands into the distribution of sero- and phage types of *Salmonella* strains found that the serovar Typhimurium was the predominant serovar in pigs between the years 1984 and 2001 (Duijkeren *et al.*, 2002). This information suggests that pigs may be one of the main reservoirs of *Salmonella* and more importantly a reservoir of the multi-drug resistant *S. typhimurium* definitive phage type DT104. Wide spread antibiotic resistance has become a serious public health risk over the last decade and multi antibiotic resistance has been reported in many food borne pathogens including *Salmonella*. Antibiotic resistance profiles for strains of *Salmonella* spp. isolated from the pork samples in Northern Ireland were determined using an agar disk diffusion technique.

The aim of this research is to:

1. Assess antibiotic resistance among *Salmonella* spp. isolated from pigmeat in Northern.
2. Generate PFGE macro-restriction fingerprints of the *Salmonella* spp. isolated from pig meat in Northern Ireland. PFGE is regarded as the "Gold Standard" for the typing and strain identification of *Salmonella* isolates (Brown *et al.*, 2006) (Tamada *et al.*, 2001).

## Material and methods

All *Salmonella* isolates were examined by the "Modified Stoke's Technique" a disc diffusion method testing isolates for sensitivity to 12 antibiotics: Amikacin (AK 30µg), Ampicillin (AMP 10µg), Apramycin (APR 15µg), Cefotaxime (CTX 30µg), Ceftazidime (CAZ 30µg), Chloramphenicol C 10µg, Ciprofloxacin (CIP 1µg), Compound Sulphonamides (S3 300µg), Furazoldidose (FR 15µg), Streptomycin (S 25µg), Sulphamethoxazole/trimethoprim (SXT 25µg) and Tetracycline (TE 10µg). PFGE macrorestriction fingerprint images are created using the PulseNet protocols (CDC, 2006). This molecular technique is used to create an individual DNA fingerprints for all *Salmonella* isolates.

## Results

Antibiotic resistance was demonstrated in 29 isolates. The antibiotics with least effect were Tetracycline (74%), Streptomycin (29%), Sulphamethoxazole/trimethoprim (26%), Compound Sulphonamides (26%) and Ampicillin (11%). Two of the *S. typhimurium* isolates displayed the ACSSuT phenotype associated with multidrug resistant *S. typhimurium* DT 104. These two *Salmonella* isolates also displayed resistance to Sulphamethoxazole/trimethoprim.

## Discussion

In this study the antibiotic resistance profiles for strains of *Salmonella* spp. isolated from the pork samples in Northern Ireland were determined using the Modified Stokes technique. Comparison of the antibiotic resistance of the salmonellae tested in this project suggested that antibiotic resistance was observed in 76% of all serovars tested. A high resistance to tetracycline (74%) was observed, this result may be attributed to tetracycline being the most common therapeutic drug used by the pork industry (Burch, 2005). The second most common therapeutic antibiotic used in the pig industry a sulphamethoxazole and trimethoprim (Burch, 2005) which also exhibited a high antibiotic resistance profile, with over a quarter (26%) of all the *Salmonella* isolated from pork in Northern Ireland displaying resistance. Ampicillin and apramycin are also commonly used therapeutically throughout the pig industry (Burch, 2005) (DEFRA, 2007). In all 18 of the 19 *S. rissen* isolates were resistant to tetracycline. This result was supported by a Spanish study that concluded that a *S. rissen* isolated from pork harboured the tet(A) resistant gene (Ioana *et al.*, 2006). A high level of intermediate resistance was observed in 6 out of the 12 antibiotics analysed in this study. This may suggest an increase in the number of antibiotic resistant *Salmonella* spp. in years to come. PFGE has become a valuable tool for the epidemiological typing of all *Salmonella* including *S. typhimurium*. The majority of multi-resistant DT104 has the distinctive *Xba*I generated macrorestriction fingerprint that can be detected by PFGE (Threlfall, 2000) (Doran *et al.*, 2005). PFGE images were generated for all the *Salmonella* isolates in this project.

## Conclusion

76% of *Salmonella* spp. isolated in this study had resistance to one or more of the antibiotics tested. These results highlight the need for continual monitoring and control of *Salmonella* in food animals.

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